Brain mechanisms for skin stroking processing in healthy subjects and anorexia nervosa patients

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Abstract

Functional magnetic resonance imaging (fMRI) allows us to study brain processing of inputs from sensory neurons both at the level of single brain areas but also at the level of larger brain networks. In this thesis we present results from two experiments, in which brain processing of gentle skin stroking in healthy subjects and patients with anorexia nervosa was investigated with this technique.

The first experiment investigated differences in brain processing of skin stroking, a caress-like stimulus that is perceived as pleasant, and skin vibration, an emotionally neutral stimulus not associated with social communication between humans. In **Paper I** we present results that show correlation between perceived pleasantness of skin stroking and neural processing in posterior supratemporal sulcus, an area previously associated with processing of biological motion in the visual domain. In **Paper II** we investigated more closely processing of stroking and vibration stimuli in insula. Insula is a paralymbic brain area previously associated with the emotional component of skin stroking and its effect on our well-being. Our results show that insula is activated both by gentle skin stroking and vibration. We performed functional connectivity analysis of these responses and proposed two paths for processing of somatosensory stimuli in insula, important for emotional and exteroceptive evaluations of touch.

In the second experiment we investigated differences in brain processing of gentle skin stroking between patients with anorexia nervosa (AN) and healthy participants. In **Paper III** we present results that show alterations in dorsal striatum and lateral occipital cortex (LOC) while skin stroking is processed in AN. Both areas have previously been implicated in the pathophysiology of AN: dorsal striatum is associated with altered eating behavior and LOC in visual processing of images of bodies. In **Paper IV** we investigated resting state functional connectivity between LOC and other parts of brain and found a coupling with medial prefrontal cortex in AN but not in healthy participants. We suggest that this coupling can be one of the brain mechanisms behind body image disturbance in AN, a finding that might be useful in the design of new therapeutic approaches.

Keywords: touch, fMRI, resting state, anorexia nervosa, posterior superior temporal sulcus, insula, lateral occipital cortex, dorsal striatum

Populärvetenskaplig sammanfattning

Funktionell magnetresonansavbildning (fMRI) är en teknik med vilken vi kan mäta hur aktiviteten i små delar av hjärnan ändras när vi utsätts för olika stimuli eller utför olika uppgifter. Dess främsta fördel är att vi kan följa dessa aktiviteter för många olika områden samtidigt, vilket ger oss möjlighet att studera hjärnans komplexa organisation och funktion. I den här avhandlingen undersöker vi hur hjärnan bearbetar stimuli i form av lätt, långsam hudstrykning.

Trots att den är en enkel stimulus, tidigare fMRI studier har visat att hudstrykning engagerar inte bara hjärnområden viktiga för vår uppfattning av beröring utan också områden som är starkt förknippade med emotioner. Baserat på dessa studier, en lång rad hypoteser om funktion av lätt hudstrykning och dess betydelse för vår utveckling och välmående har lagts fram. I första delen av avhandlingen testar vi två av dessa hypoteser i ett fMRI experiment på friska försökspersoner.

I den andra delen av avhandlingen undersöker vi om det finns skillnad i hjärnans bearbetning av lätt hudstrykning mellan friska personer och patienter med anorexia nervosa (AN). En av de främsta kännetecknen av AN är störd kroppsuppfattning, som manifesteras genom att dessa patienter upplever sig själva som tjocka trots den uppenbara utmärglingen. Tidigare fMRI studier av kroppsuppfattning har varit fokuserade på betydelsen av hur hjärnan svarar på bilder av människokroppar. Med vår studie undersöker vi hur AN patienter svarar på kroppsberöring.

Våra resultat från den första delen av avhandlingen visar en mera komplex bild av hjärnans bearbetning av hudstrykning än vad som tidigare föreslagits. Vi visar att den upplevda behagligheten av hudstrykningen korrelerar med aktiviteten i en del av hjärnan som är viktig för vår förmåga att visuellt urskilja biologiska rörelser, t ex att vi kan föreställa oss en människa som rör sig även när denna rörelse presenteras av en rad punkter lokaliserade på olika delar av människokroppen. Detta fynd demonstrerar en viktig princip av hur hjärnans funktion är organiserad: för bearbetning av mera komplexa komponenter av olika sensoriska stimuli, i det här fallet beröring och syn, använder hjärnan samma områden för egenskaper med liknande innebörd. I det här fallet kan hudstrykningar associeras med smekningar och människorörelser. Vi visar dessutom att de delar av hjärnan som tidigare identifierats som centra för emotionell bearbetning av hudstrykningar svarar också på beröringsstimuli som inte uppfattas som behagliga eller obehagliga. Skillnaden mellan behagliga och neutrala sti-

muli uppträder först när vi undersöker hur dessa områden kommunicerar med varandra när stimuli presenteras. Detta demonstrerar en annan princip viktig för vår förståelse av hjärnans funktion: information som når hjärnan via sensoriska nervceller aktiverar inte enskilda hjärnområden var för sig utan snarare nätverk uppbyggda av centra i olika delar av hjärnan.

Resultat från vår studie av AN patienter pekar mot störning i ett sådant nätverk. Vi visar att svaret på lätt hudstrykning är annorlunda i denna patientgrupp jämfört med friska personer i ett hjärnområde som ligger i bakre delen av hjärna. Detta område aktiveras starkt när vi tittar på bilder av kroppar och kroppsdelar men mindre när vi tittar på bilder som föreställer olika objekt. Tidigare studier har visat störningar hos AN patienter i samma del av hjärnan när de tittat på bilder av människokroppar. Att vi ser liknande störning även när vi stimulerar huden kan vara en konsekvens av att detta område tillhör ett större nätverk viktig för vår kroppsuppfattning, som aktiveras både av visuella stimuli och beröringsstimuli. Dessutom visar våra resultat att detta område kommunicerar med områden i främre hjärnbarken hos AN patienter men inte hos friska personer. Främre hjärnbarken är involverad i processer som självuppfattning och självvärdering. Det är frestande att dra slutsatsen att den här kopplingen ligger bakom den negativa effekten som kroppsutseende har på hur AN patienter ser på och värderar sig själva. Dock krävs det flera fMRI studier på denna patientgrupp för att bekräfta våra fynd och värdera dess betydelse för nya behandlingsstrategier.

List of papers

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Davidovic M**, Jönsson E, Olausson H, Björnsdotter M.

Posterior superior temporal sulcus responses predict perceived pleasantness of skin stroking

Frontiers in Human Neuroscience 2016: 10; 432.

II. **Davidovic M**, Starck G, Olausson H.

Processing of affective and emotionally neutral tactile stimuli in the human insular cortex

Manuscript

III. **Davidovic M**, Karjalainen L, Starck G, Wentz E, Björnsdotter M, Olausson H.

Abnormal gentle touch processing in anorexia nervosa

Manuscript

IV. **Davidovic M**, Karjalainen L, Starck G, Wentz E, Olausson H, Björnsdotter M.

Resting state functional coupling between lateral occipital and medial prefrontal cortex in women with anorexia nervosa

Manuscript

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Abbreviations

AN	anorexia nervosa
BOLD	blood oxygenation level dependent
CEN	central executive network
CSF	cerebrospinal fluid
DMN	default mode network
fMRI	functional magnetic resonance imaging
GLM	general linear model
GM	grey matter
LOC	lateral occipital cortex
MNI	Montreal Neurological Institute
MPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
NMV	net magnetisation vector
OFC	orbitofrontal cortex
pgACC	pregenual anterior cingulate cortex
pSTS	posterior superior temporal sulcus
RF	radiofrequency
ROI	region of interest
SN	salience network
VAS	visual analogue scale
WM	white matter

1. Introduction

1.1 Magnetic resonance imaging

Magnetic resonance imaging (MRI) is based on the phenomenon that magnetic nuclei placed in a magnetic field can absorb and re-emit energy. Even though the energy exchanged in this process is very small, the coincidence that the hydrogen nucleus is both the most abundant atom in the human body and one with strong magnetic properties has made this technique sensitive enough to produce a variety of soft tissue contrasts for structural and functional studies of the human brain. By providing whole brain images at high resolution and by its ever-increasing availability, this non invasive technique has become one of the main experimental tools for testing hypotheses about brain function in different groups of subjects, spanning all ages and cognitive abilities. MRI gives the studies of pathophysiological processes behind psychiatric diseases a new dimension: with a detailed knowledge about structural and functional changes in patiens, novel diagostic methods and therapeutucal approaches can be developed.

MRI principles

The magnetic properties of hydrogen nucleus are defined by the charge and the spin of it single constituent – proton. According to the laws of electromagnetic induction, spinning motion of a proton around its axis, characterized by the angular momentum $\boldsymbol{I}$, results in a magnetic moment $\boldsymbol{\mu}$. Being a vector quantity, magnetic moment possesses size, orientation and direction. The size and direction of the magnetic moment are determined by the equation $\boldsymbol{\mu}=\gamma\boldsymbol{I}$, in which the constant of proportionality γ is called *gyromagnetic ratio*.

When brought under the influence of an external static magnetic field $\boldsymbol{B_0}$, magnetic moments of the spinning protons experience two effects, both important for the MRI. The first one can be described using the tools of classical physics: the external field exerts a force on the spinning proton that pushes the axis of spinning to align with the direction of the magnetic field. The result of this force is a rotational motion of the spinning axis itself, which is called precession. The linear relationship between the frequency of the precession of the magnetic moment $\boldsymbol{\mu}$ and the strength of the static magnetic field $\boldsymbol{B_0}$ is expressed by the Larmor equation: $\boldsymbol{\omega_0} = \gamma\, \boldsymbol{B_0}$. The frequency of precession is referred to as Larmor frequency. When the magnetic resonance image of the brain is calculated, magnetic resonance signal is located to different single volume elements or voxels by the use of small magnetic gradients that are superimposed on the magnetic field

$\boldsymbol{B_0}$. The position of protons within a voxel is encoded by their differences in Larmor frequency.

The second effect of the field $\boldsymbol{B_0}$ is based on a quantum mechanical property of the subatomic particles, according to which there are only two permitted directions for the spinning of protons. In the absence of the external magnetic field, there is no preferable orientation for the axis of spinning and thus magnetic moments are randomly oriented. However, in the static magnetic field $\boldsymbol{B_0}$, magnetic moments of protons orient with the field and adopt one of the two allowed directions: parallel or antiparallel to the external field. These two directions correspond to two states, often referred to as 'up' state and 'down' state, where the state parallel with the field has lower energy than the antiparallel state. The energy difference between 'up' and 'down' states can be calculated from the equation:

$$\Delta \mathrm{E} = \frac{\gamma \times B_0}{2\pi} = \frac{\omega_0}{2\pi}$$

The energy difference between two states for a hydrogen nucleus in 3T magnetic field is approximately 100 MHz. In other words, the energy needed to bring a hydrogen nucleus from 'up' to 'down' state belongs to the radio-frequency range of the electromagnetic radiation spectrum. This frequency defines the energy unit or the energy 'package' necessary for nuclei to change their state.

If we were to observe magnetic moments of all hydrogen nuclei in a sample, e.g. a human brain, placed in the magnetic field of an MRI camera, we would find them almost equally split between 'up' and 'down' directions. The two states are populated according to the Boltzmann distribution, and since the energy difference is very small, the difference in the population is only approximately 1 in 10 000 (for every 10 000 nuclei in the 'down' state, there are 10 001 nuclei in the 'up' state). For this reason, MRI signal is extremely weak and can be increased only by either applying a stronger field or using contrasts with high gyromagnetic ratios.

The sum of all magnetic moments in a sample gives a *net magnetization vector* (NMV). At the beginning of an MRI experiment, NMV is aligned with the field $\boldsymbol{B_0}$. During the course of an experiment, a second field $\boldsymbol{B_1}$ is applied tailored exactly to change both the size and the direction of the NMV. Unlike $\boldsymbol{B_0}$, which is static, $\boldsymbol{B_1}$ is an oscillating magnetic field whose frequency of oscillations matches the Larmor frequency $\boldsymbol{\omega_0}$. In addition, $\boldsymbol{B_1}$ is transient lasting usually few seconds, and is therefore called a radiofrequency (RF) pulse.

The effect of an RF pulse on the hydrogen nuclei (protons) is twofold. Firstly, RF pulse supplies nuclei with energy 'packages' for them to go from the lower energy state to the higher energy state – a phenomenon called *resonance*. By changing the intensity of the RF pulse, the number of energy 'packages' and thus

the population of the two states can be controlled. In this way, NMV component aligned with the field $\boldsymbol{B_0}$ is changed. The second effect of the RF pulse concerns the precessions of magnetic moments. Magnetic field that oscillates with frequency $\boldsymbol{\omega_0}$ forces all magnetic moments in the sample to precess in phase - a phenomenon called *phase coherence*. These two effects give rise to an NMV component in the plane perpendicular to direction of the static field $\boldsymbol{B_0}$, which is measured as MR signal.

When RF pulse is switched off, NMV returns to its starting position and an exponential decay of the MR signal is observed. Time constant of the exponential decay is governed by a process in which the excited nuclei release energy supplied by the RF pulse. This process, called *relaxation,* depends on two mechanisms. The first is called spin-lattice or T_1 relaxation: energy 'packages' are taken up by the molecular motion ('lattice'). Molecules can display different motions, i.e. translational, rotational and vibrational, and each of them is associated with different ranges of energies. In fMRI experiments, the main source of T_1 relaxation is rotational motion (tumbling) of molecules. The energy of the rotational motion decreases with the increasing size of the molecules at room temperature, and approaches the size of the energy 'packages' necessary for T_1 relaxation, in other words tumbling frequency approaches Larmor frequency. When we measure T_1 relaxation in biological tissues we can observe that tissues containing large, densely packed molecules have higher T_1 values (i.e. relax faster) than compartments filled with water solutions of smaller molecules.

The second relaxation process is called spin-spin or T_2 relaxation: the population of the precessing nuclei lose their phase coherence imposed on them by the RF pulse. Although mutual interaction between the spins is the basis for T_2 relaxation, this process is sped up by local inhomogeneities in the magnetic field and if taken into account the characteristic time for the second relaxation process is denoted as T_2^*.

The dependency of T_1 and T_2 relaxation on the properties of the tissue in which protons reside is the basis of image contrast, e.g. we can differentiate grey matter from white matter in the brain. In white matter (WM), protons are surrounded by relatively slowly tumbling fat molecules and the relaxation is relatively fast. In grey matter (GM), protons are surrounded by faster tumbling water molecules and relaxation is slower.

Blood oxygenation level dependent contrast

Blood oxygenation level dependent (BOLD) contrast is based on the phenomenon that iron atoms in the oxygenated and deoxygenated hemoglobin molecules have different magnetic susceptibilities. When non-magnetic materials are placed in the static magnetic field, they acquire magnetic properties and create small local magnetic fields directed either with the external magnetic fields (paramagnetic) or opposite to it (diamagnetic). Most tissues are diamagnetic, i.e.

they create a small opposing magnetic field when placed in the magnet camera. The differences in magnetic susceptibilities create inhomogeneities in the magnetic field at the interfaces of structures like air, bone and other tissues. Inhomogeneities in the static magnetic field lead to the increase of T_2^* and faster decay of the MR signal, an effect known as susceptibility artifact.

Hemoglobin contains four polypeptide subunits, each coupled to one iron ion via protoporphyrin complex. When oxygenated, hemoglobin has diamagnetic properties and in this respect resembles the majority of the body tissues. However, upon the release of oxygen molecules, two additional electrons in the iron ions become unpaired. In this state iron ions are paramagnetic. This change from diamagnetic to paramagnetic properties causes small changes in the homogeneity of the local magnetic field which we can capture in our measurements of T_2^*.

In functional MRI (fMRI), T_2^* relaxation times in the brain are measured during activity or stimulus and rest. The local increase in the level of oxygenated hemoglobin and decrease in the level of deoxygenated hemoglobin lead to change in the local field inhomogeneity, resulting in a change in T_2^* relaxation time and BOLD contrast (1). A regional increase in the local levels of oxygenated hemoglobin is a consequence of both regional increase in cerebral blood flow and volume, i.e. hemodynamic response due to the neurovascular coupling, and the limitation of oxygen consumption by diffusion (2). This is the mechanism behind positive BOLD signal. Since the increase in BOLD signal correlates with the change in local field potentials, it is suggested that an increase in the BOLD response reflects the input and intracortical processing of neural signals rather that the spiking output (3). It is also shown that negative BOLD signal arises due to a decrease in the inflow of oxygenated blood, which leads to the depletion of oxygen supply and an increase of the level of deoxygenated hemoglobin (4).

The functional specificity of brain regions is investigated using task fMRI experiments. Usually, regional increase in the BOLD response is measured applying different paradigms and by subtracting the levels of BOLD responses during different tasks or stimuli an inference about the function of brain regions is drawn. This cognitive subtraction approach is based on an assumption about pure insertion. However, it has early been recognized that the regional neural processing depends also on the interaction among the cognitive components of a task (5) and modulation from other brain regions (6). These effects can be investigated by a method of psycho-physiological interaction (PPI) (6). PPI measures context-specific functional connectivity between brain regions associated with the cognitive processing.

Resting state fMRI is an approach that measures spontaneous, low-frequency (<0.1 Hz) BOLD fluctuations in the resting brain. Temporal correlations of spontaneous fluctuations in BOLD signal in the somatomotor cortex were first reported by Biswal et al 1995 (7). Subsequent studies have described several networks of brain areas with high correlations in slow, spontaneous fluctuations

in healthy subjects (8–11), e.g. default mode network (DMN), central executive network (CEN) and salience network (SN).

DMN consists of a set of brain areas located in ventral medial prefrontal cortex, posterior cingulate cortex and bilateral lateral posterior parietal cortices (Raichle, 2015). In addition to the high functional connectivity in resting state fMRI experiments, it is characterized by decreased activity level during task fMRI experiments. In addition, it is observed that DMN and CEN are anti-correlated in resting date data. A decrease in the DMN during cognitive tasks depends on the task difficulty and is most pronounced in attention-demanding, non-self-referential tasks. The decrease in DMN and increase in CEN during task performance is one example of network organisation of brain activity that maintains energy consumption balance (9,12,13).

1.2 Gentle skin stroking

It is widely recognised today that the sense of touch is not only important for the exploring of the world that surrounds us, but also has affective and social components. Sense of touch arises through the direct interaction between the external world and our skin, and we respond to this interaction in different ways. The primary goal of touch is to identify objects, by exploring their shape, size, weight, texture and other physical properties, referred to as discriminative quality of touch. Affective and social aspects of touch are more complex, and depend not only on the primary tactile input but also on the relationship with the person we encounter, situation and context in which it occurs, etc. Positive, hedonic experience of touch is associated with behaviours such as hugging, kissing and caressing in humans, and grooming and licking in animals. Positive effects of touch for our well-being are well documented.

Advances in neuroimaging techniques during past two decades, fMRI in particular, have made it possible to study brain representations of touch processing in humans. So far we have learned that brain processing of touch information is complex and involves many brain areas outside the somatosensory cortex. In the first part of this thesis we continue to explore the brain mechanisms for touch perception in healthy participants, with the focus on affective touch. We use gentle skin stroking as a tool in this endeavour. Gentle skin stroking has several advantages. It is a caress-like touch associated with a pleasant sensation, as shown in many experiments. Skin stroking is easily handled in the magnet camera setting and can be contrasted with a variety of other somatosensory stimuli, which opens the possibility to study the processing of pleasant touch by different tactile paradigms addressing different hypotheses. These experiments can easily be repeated which is important for the tests of reproducibility of the results. In addition, we already have a substantial body of knowledge on both the central and peripheral processing of skin stroking derived from previous experiments.

1.2.1 Skin as a sensory organ

Richness of the perceived tactile input is provided by a system of mechanoreceptors situated in the skin. Each time we touch the skin, we stimulate a group of these mechanoreceptors, which transform the mechanical energy of the touch into the electric energy of the action potentials (spikes). All mechanoreceptors that constitute a tactile perception system respond to skin indentation with low forces and are correspondingly called low threshold mechanoreceptor. In the glabrous skin, which covers palms and feet, they are composed of four types of organs with well characterized structures and functions, all supplied by thick, myelinated afferents with fast conducting velocities. They are classified according to their response to prolonged skin indentation in: slowly adapting type 1 afferents associated with Merkel cells; slowly adapting type 2 afferents associated with Ruffini endings; rapidly adapting type 1 afferents associated with Meissner corpuscles; and rapidly adapting type 2 afferents associated with Pacinian corpuscles (14,15). Mechanoreceptors associated with type 1 afferents are situated in the superficial skin layers at the margin between dermis and epidermis and are densely distributed with small receptive fields. Mechanoreceptors associated with type 2 afferent reside in deeper layers of dermis and subcutaneous tissue and are more sparsely distributed with large receptive fields.

Mechanoreceptors of hairy skin in humans remain to be characterised in detail, but based on the animal studies it is suggested that, in addition to Merkel cells, hair follicles play role in mechanosensation (16). Sensory afferents that respond to low thresholds of mechanical stimulation are on the other hand well described. They comprise both myelinated and non-myelinated neurons, with slowly (type 1 and 2 myelinated neurons), intermediate (non-myelinated tactile neurons) and rapidly (hair and field myelinated neurons) adapting properties.

Our understanding of the properties and specificity of sensory neurons that innervate hairy skin in humans, C tactile sensory neurons in particular, relies on the experiments performed with a technique called microneurography.

C tactile neurons in humans were first discovered in the microneurography experiment on the supraorbital nerve (17). They differed from other sensory neurons by their slow conducting velocities (around 1 m/s) and very low thresholds for indentation (around 1mN). They responded to sustained skin indentation with high initial peak firing rates and rapid decrease in firing, with an after discharge when the stimulus is released. Responses to gentle skin stroking were also measured. Slow skin stroking (about 0.5-1 cm/s) was particularly effective, resulting in the peak firing rates up to 100 imp/s. Faster stroking (about 5-10 cm/s) produced both lower firing rates and lower number of spikes. Repeated stimulation resulted in the decrease of firing rates after approximately four repetitions demonstrating receptors a tendency to fatigue.

Several experiments followed (18,19) and confirmed that C tactile neurons indeed fire with high peak firing rates and a large number of spikes when stimulated with slow (0.2 and 0.4 cm/s), light (40 mN) stroking. Faster skin stroking (1 cm/s) also resulted also in high peak firing rates although the number of spikes was reduced dramatically. It is again noted that slow stroking stimulus resulted in peak firing rates just below 100 imps/s, which at the time was considered as the maximal rate achievable by non-myelinated neurons. Measurements were done on antebrachial cutaneous nerve and C tactile neurons were here fairly common. An experiment that investigated receptive fields of C tactile neurons (20) showed a large variation in the size and number of sensitive spots. In addition, sensitive spots of the same receptive field could fire with different rates when stimulated with same stimulus. Neurons fired both when moving stimulation was applied in proximal-distal and distal-proximal direction.

The seminal paper about C tactile neurons was published in 2009 (21). Here, the authors investigated responses to gentle skin stroking in all low threshold sensory neurons in hairy skin, with a focus on responses to different stroking velocities. The range of velocities that were tested can be divided into slow (below 1 cm/s), intermediate (1-10 cm/s) and fast (above 10 cm/s) velocities. Analysis of the spike trains was done with respect to total number of spikes and mean firing rates. However, no peak firing rates were presented. C tactile neurons fire with a large number of spikes at slow velocities (up to 150), this number decreases for intermediate velocities (to around 25) and continues to decrease for fast velocities. Mean firing rates however follows an inverted U shape: it has values around 25 imp/s at slow velocities, reaches 50 imp/s for intermediate velocities and goes down for fast velocities. For slowly adapting afferent, the number of spikes shows an exponential decrease with increasing velocities: it is around 150 at lowest velocity and goes down to few spikes at highest velocities. Their mean firing rate increases instead exponentially with increasing velocities: it starts with very low values at low velocities and goes up to around 250 imp/s for high velocities. For hair and field afferents, the number of spikes at low velocities is somewhat lower than for C tactile and slowly adapting neurons, with values between 50-10, and it decreases to lower values at higher velocities. Their mean firing rate shows similar velocity dependence as slowly adapting afferents. Since the velocity dependence of perceived pleasantness ratings of skin stroking displays inverted U shape and thus correlates with the mean firing rate of C tactile neurons, it is concluded that the neural coding of C tactile neurons is tuned to the pleasant skin stroking. It is further concluded that the velocities in the intermediate range are C tactile neurons' optimal velocity. This has had a major impact on the research of central processing of skin stroking which became focused on the intermediate velocities.

1.2.2 Central processing of gentle skin stroking

Axonal terminals of both myelinated and non-myelinated sensory neurons project to the dorsal horn of the spinal cord, myelinated in lamina III-V and non-myelinated in lamina I-II. Here, the first integration of tactile input occurs (16). Second order neurons that carry the information from myelinated neurons follow the dorsal column path towards brain, referred to as indirect path. Some of the myelinated sensory neurons, primarily from the glabrous skin of hand, do not project to the dorsal horn but take the dorsal column path directly (16). After synapsing in dorsal column nuclei and decussation in medial lemniscus, the signal from myelinated neurons reaches thalamus. C tactile neurons' path from spinal cord has not yet been determined. Since they are non-myelinated and terminate in lamina I-II it is suggested that they follow spinothalamic path like other thinly- or non-myelinated afferents, i.e. thermo- and nociceptors (20,22,23).

Brain processing of the input from neurons activated by gentle skin stroking is complex. Primary somatosensory cortex is the main target for the input from myelinated sensory neurons. Whether the information from C tactile sensory neurons is processed by primary somatosensory cortex or not is difficult to study because in healthy subjects it is not yet possible to selectively activate C tactile neurons. Our main insight into the brain processing of these neurons comes from experiment on two subjects who, due to peripheral neronopathy, lost functions mediated by myelinated sensory neurons but have all non-myelinated neurons intact, C tactile neurons included. fMRI experiments with skin stroking paradigm in the intermediate velocity range reveal activations in the posterior insular cortex contralateral to the side of stimulation, but no activations in the primary or secondary somatosensory cortices in both patients (23,24). In addition, one of the patients shows activations in bilateral anterior insula. Interestingly, on the whole brain level, this patient showed the highest level of activity (t=7.3) in the contralateral retroinsular cortex.

The next study important for further association between the skin stroking at intermediate velocities, C tactile neurons' activity and the posterior insular cortex investigated somatotopic organization of gentle skin stroking input in posterior insula. This was done using fMRI data from skin stroking paradigm with intermediate velocities, where stimuli were applied on the forearm and thigh (22). Both healthy subjects and one of the neuronopathy patients were included. Data showed that the peripheral input from skin stroking stimuli to the posterior insula is indeed somatotopically organized, which is taken as an indication that C tactile neurons have primary input from thalamus to posterior insula.

Based on the experiments presented so far, it is proposed that the main function of C tactile neurons is not to communicate discriminative properties of touch. Due to their slow conducting velocity, irregular receptive fields and correlation

of mean firing rates with the perceived pleasantness of skin stroking, C tactile neurons are described as a parallel component of the peripheral tactile system, devoted to positive hedonic perception of touch. Furthermore, since the connection between C tactile neurons and activation in posterior insula was established, it is suggested that these neurons selectively relay information that is processed not by somatosensory cortex but rather by paralimbic and limbic brain areas. Following the view developed by Bud Craig, which includes termo- and nociceptors into the system of interoception (25,26), a hypothesis about the C tactile neurons as a class of skin's interoceptive neurons was put forward (22,27,28). Thus, it is suggested that C tactile neurons not only mediate the pleasantness of skin stroking, but also contribute to our state of well-being.

Another hypothesis about the role of C tactile neurons comes from the observation that their mean firing rate reaches maximum at the skin stroking velocities that occur in interpersonal communication. This hypothesis is known as the social touch hypothesis (28–30). It is suggested that information that reaches the brain via C tactile neurons is important not only for social communication but also for the development of cortical social circuits.

Several neuroimaging studies followed, focused on the search for neural correlates of these processes. Two studies used combination of visual and tactile stimuli. In one of them, participants were presented with both skin stroking at intermediate and fast velocities but also with videos of others' arm being stroked at the same velocities (30). Posterior insula was sensitive not only to perceived pleasant touch but also to the same touch when presented in the visual domain, i.e. it activated when participants watched videos presenting other persons' arm being stroked. In addition, when contrasted with fast velocities, and for combination of tactile and visual stimuli, significant activity for intermediate skin stroking was observed only in the posterior insula at the whole brain level ($p<0.001$ and cluster size>50). In a second study, participants viewed tactile stimuli with different emotional valences, among them caresses (31). Participants also received skin stroking stimulus in a separate session. Interestingly, the effect on posterior insula with this paradigm was opposite: while skin stroking resulted in an increase in activity, viewing caresses was followed by a decrease in activity compared with the baseline. Overlap between tactile and visual stimuli in this study was observed in the bilateral secondary somatosensory cortex, bilateral posterior middle temporal gyrus (pMTG) and right posterior superior temporal sulcus (pSTS), among other areas. It is important to note that posterior insula showed decrease in the activity when participants viewed tactile stimuli with both positive and negative emotional valence, and that only one stroking velocity was tested.

A PET study, in which participants received gentle skin stroking at intermediate and fast velocities, on both glabrous skin of the palm and hairy skin of the forehand, found that orbitofrontal cortex (OFC) was activated specifically for intermediate velocities and hairy skin stroking (32). Interestingly, for the same touch,

performing a region of interest analysis the authors found activations in both posterior insula and retroinsular area previously reported for one of the neuronopathy patients (23). Finding of activation in OFC is consistent with the results in one of the earliest fMRI experiment of gentle skin stroking on hairy skin by Frances et al, 1999. Recently, an fMRI study investigated similar paradigm as in the aforementioned PET study, i.e. both intermediate and fast velocities were used, on both the palm and the back of the hand (33). In addition, participants rated pleasantness and intensity of all tactile stimuli. When contrasting intermediate with fast velocities for the hairy skin, a significant difference was observed in pregenual anterior cingulate cortex (pgACC). Also, activity in this area correlated with the pleasantness ratings, when correlation was estimated for all four types of tactile stimuli combined, i.e. both velocities and both skin sites. Another study investigated brain responses to touch on the forehand delivered by hand, i.e. direct skin to skin contact, and showed that only stroking by hand activated pgACC (34). This was not observed for stationary touch or stroking at intermediate velocities with a rubber glove. The importance of skin-to-skin contact for C tactile neurons' firing is further demonstrated by a microneurography experiment, which shows decreases in mean firing rates over whole velocity range when temperatures of touching objects increase or decrease from 32 ^{o}C (35). pgACC and OFC were also observed in a study that investigated cognitive modulation of perceived skin stroking (36). OFC was found for the contrast skin stroking on the hairy skin versus hand while pgACC correlated with the cognitive modulation. These studies together highlight the importance of the prefrontal cortex for the perception of affective properties of skin stroking stimuli.

Three neuroimaging studies aimed to investigate the effects of gentle skin stroking on the areas important for the processing of social cues. Two fMRI studies found increased activity in pSTS when skin stroking with intermediate velocity was applied on the hairy skin but not on the palm (37) or with fast velocities (38). A subsequent functional near-infrared spectroscopy (fNIRS) study confirmed that pSTS indeed responds to gentle skin stroking on the hairy skin (39). Posterior temporal lobe contains two areas that are important for the perception of motion: pSTS and pMTG. The processing of visual stimuli in these areas is well characterized: while both pSTS and pMTG respond to a range of moving stimuli, only pSTS shows sensitivity towards biological motion, i.e. articulated motion of human bodies (40). It is also shown that both areas increase their response when both visual and congruent auditory information is presented, demonstrating the multimodal level of processing in these areas (41). pSTS is also part of a brain network involved in the recognition of facial expressions of emotions, both in the visual domain but also when faces are haptically explored (42).

In the first part of this thesis we investigate responses in pSTS and insula with a new paradigm (**Paper I** and **Paper II**). We contrast gentle skin stroking with vibration stimulation. We use the same velocity for the skin stroking as in previ-

ous experiments. The question we aim to answer is if there are differences between the processing of stroking and vibration in these two brain areas.

1.3 Anorexia nervosa

Anorexia nervosa (AN) is a severe psychiatric condition predominantly affecting young women (43). AN is characterized by preoccupation with control of eating, weight and shape, and with an intensive fear of gaining weight (44). For individuals with AN, the preoccupation with shape and weight affects the evaluation of self-worth, leading to a disturbed body image and significantly low weight (44). There are no evidence-based treatments for adults with AN (45), and studies evaluating treatment effectiveness for adults are scarce (46). Half of the adult AN patients recover and one in five develops chronic AN (47). For adolescent patients, however, family-based therapy is regarded as the gold standard (45). In order to apply new therapeutic approaches, we need to better understand the mechanisms behind the symptoms observed in AN.

The maintenance of AN can be neurobiologically understood by considering disruptions in three systems: the homeostatic system (in the brain stem and hypothalamus) which regulates hunger and satiety, the drive system (mesolimbic cortex and striatum), with afferent input from sensory organs implicating learning and memory, and the self-regulating system connecting appetite to goals, values and meanings (48). Cultural, social, and interpersonal factors may trigger disease onset, and changes in neural networks can sustain the illness (48). AN may also be explained by disturbances in the ventral limbic circuit (amygdala, insula, striatum and ventral medial prefrontal cortex), which identifies emotion salience and contributes to affective states, and the dorsal executive circuit (dorsal anterior cingulate cortex, dorso-lateral prefrontal cortex and parietal cortex), which is important for selective attention and planning (49).

Given its complexity, AN is probably best investigated by methods that target connectivity between brain areas (50). It has been suggested that the studies of alterations in resting state fMRI functional connectivity may unravel the functional changes in neural networks that underpin the neurobiology of the psychiatric disorders (50). The vast majority of resting state fMRI studies in AN patients searched for disturbances at a network level by analysing resting-state data using independent component analysis (ICA). These studies show alterations in the visuospatial and somatosensory resting state networks (51); increased functional connectivity between the precuneus and dorsolateral prefrontal cortex, and alterations in the default mode network (DMN) (52); and increased functional connectivity between the angular gyrus of the left fronto-parietal network, and the anterior insula and frontal operculum belonging to the DMN (53).

Anorexia nervosa and alterations in the tactile perception

Several psychophysical studies have investigated discriminative touch in AN. Testing for two-point discrimination shows that patients with AN overestimate distances between tactile stimuli on both the arm and abdomen. In the same experiment, patients visually overestimated the images of their bodies (54,55). Another experiment tested the perception of tactile stimuli applied with different orientations along the body axis (56), and showed that the AN patients judged horizontal tactile stimuli significantly wider than the same stimuli oriented vertically. Together these studies show that the body image disturbance in AN is widespread and encompasses not only visual but also somatosensory perception. Hence, it is suggested that the disturbance lies in the higher-level processing of the sensory information. A recent study investigating affective touch in AN patients shows that they perceive stroking at intermediate velocities as less pleasant compared with the group of healthy controls (57), demonstrating a disturbance also in the emotional processing of skin stroking stimuli.

In the second part of this thesis we use gentle skin stroking as a tool to investigate alterations in brain circuits for processing of sensory information in patients with AN. Skin stroking results in strong input for both myelinated and C tactile peripheral sensory neurons, and opens a possibility to study brain mechanisms behind the alterations previously observed for both discriminative and affective properties of touch in AN patients. In **Paper III** we aim to find brain areas with altered responses to gentle skin stroking in AN. In **Paper IV** we further explore resting state functional connectivity of areas identified in Paper III, with the goal to identify circuits associated with the pathophysiology of the disease.

2. Aims

fMRI studies of brain mechanisms important for processing of affective and social information are constrained by the environment of the magnet camera. However, due to its availability and since it is not harmful to the participants, we can repeat fMRI experiments many times and with small variations in paradigms that put new perspectives on the interpretation of the results from previous experiments. Previous studies have tested brain processing of gentle skin stroking using paradigms which contrasted either different stroking velocities (i.e. slow versus fast) or different skin sites (i.e. hairy versus glabrous skin). Here, we use a new paradigm in which we contrast skin stroking with skin vibration. While skin stroking contains both a discriminative and an affective component, skin vibration is mainly discriminative. In addition, vibration is an artificial type of touch and it is less associated with social processes. In **Paper I** we investigate brain responses to these two tactile stimuli in pSTS, an area important for processing of visual social cues and as well as gentle skin stroking. In **Paper II**, we focus our analyses on insula and ask whether the insular activation is specific to the affective tactile stimulus or if it also responds to a tactile stimulus with less emotional valence. In the second part of this thesis we investigate brain processing of gentle skin stroking in AN patients. Previous studies show that AN patients have alterations in perceiving both discriminative and affective touch. In **Paper III** we investigate the brain responses to gentle skin stroking in a group of AN patients and a group of healthy controls. In **Paper IV** we further investigate alterations observed in Paper III, using functional connectivity analyses.

In summary, specific aims were to answer the following questions:

1. Does pSTS specifically respond to the pleasant skin stroking?
2. Does insula process both affective and emotionally neutral tactile stimuli?
3. What are the brain mechanisms for altered tactile perception in AN?
4. Are alterations in the brain processing of tactile information in AN associated with the disturbances in communication between brain areas important for the pathophysiology of the disease?

3. Methods

3.1 Ethics and participants

The studies are approved by the Regional Ethical Review Board at the University of Gothenburg, Sweden (Dnr: 890-13 and 007-14).

Healthy participants were recruited from universities and high schools in Gothenburg, Sweden. Women with AN were recruited consecutively from an in- and outpatient specialist unit, the Anorexia-Bulimia unit at the Queen Silvia Children's University Hospital. The group of healthy controls included age matched women. All participants received a monetary compensation, in accordance with the ethical approval.

3.2 Stimuli and imaging paradigms

In the design of the experiment in which we compared skin stroking with vibration (**Paper I** and **Paper II**) we were guided by the design presented in Morrison et al, 2011 (30). We used similar block design but instead of two stroking velocities we used skin stroking at the velocity 2 cm/s alternated with vibrations at 100 Hz. Tactile stimuli were delivered by hand, on the right dorsal forearm. We excluded visual stroking stimuli (30). Instead we added one block during which a visual analogue scale (VAS) was shown and participants were asked to rate the pleasantness of the last stimulus, both stroking and vibration. We modified also the task (30). While in the previous study the participant were asked to detect either the repetition of stroking direction or diagonal deviation of the stroking, the task in our experiment was to focus on the affective property of the tactile stimulus, i.e. whether it felt pleasant or unpleasant. Scanning sessions included one resting state session, one tactile task session and one T_1–weighted scan as anatomical reference. During the resting state session, which lasted 10 minutes, subjects were instructed to keep their eyes closed, think of nothing in particular, and not to fall asleep. The task session lasted 12 minutes.

For the tactile experiment in the second part of this thesis (**Paper III** and **Paper IV**) we used a custom-built robotic linear tactile stimulator to deliver stroking. In this way, we limited any potential experimenter confounds and assured that all participants received identical tactile stimuli. The robotic stimulator applied high precision brush strokes at a velocity of 2 cm/s to the skin of the right dorsal forearm. The periods of brushing were alternated with the periods of static touch, i.e.

brush was gently touching the skin but not moving. Each stimulus lasted 8 s. Participants were instructed to focus on the affective properties of the received skin stroking. One task session consisted of 10 cycles of stroking/static touch stimuli, which was repeated four times. One task session lasted 3.5 minutes. At the end of each task session the participants were asked to rate verbally the pleasantness of the stroking on a scale between –5 and 5, with the endpoints 'unpleasant' and 'pleasant'. The experiment included one T_1–weighted scan, one resting state scan and four task scans in this order. During the resting state scan participants were instructed to look at a fixation cross, to think of nothing in particular, and not fall asleep. The resting session lasted 6.5 minutes.

3.3 MRI acquisition and data preprocessing

All experiment were performed on Philips Gyroscan 3T Achieva, software release 3.2, (Philips, Eindhoven, The Netherlands), at Sahlgrenska University Hospital.

Preprocessing and statistical analysis of MRI data were performed using the SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Anatomical images were segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) images. GM images were used to determine the 12-parameter affine transformation into standard stereotactic space (Montreal Neurological Institute, MNI). Preprocessing of functional task and resting-state images included slice time correction, realignment to the first volume of the first run (using a 6-degree rigid spatial transform), co-registration to anatomical images, transformation to MNI space (using 12 parameters obtained from the transformation of GM images), resampling to voxels 2x2x2 mm and smoothing with 6-mm full width at half maximum Gaussian kernel. In addition, motion artefacts were examined using the Artefact Detection Toolbox. Volumes in which the global signal deviated more than two standard deviations from the mean signal or in which the difference in motion between two neighbouring volumes exceeded 1 mm (across rotational or translation directions) were marked as outlier volumes. Smoothed functional images were filtered with 128 s high pass filter.

3.3 Analysis of task data

For the analysis of fMRI data we used the general linear model (GLM) approach (58). At the single subject level, the measured BOLD signal is represented by the linear combination of a set of regressors and noise, and the analysis consists of finding a set of parameter estimates (betas) for each regressor that minimizes the sum of squares of residuals. Regressors are obtained by the convolution of model functions with a hemodynamic response function, and represent the predicted

BOLD responses corresponding to each task condition. The analysis is performed in a mass univariate fashion, i.e. for each voxel independently.

For the task data from the first experiment (**Paper I** and **Paper II**) we defined four model functions corresponding to four conditions (stroking, vibration, VAS rating for stroking and VAS rating for vibration) using a boxcar function with 1 during the 15 s stimulus conditions and 0 otherwise. For the task data in the second experiment (**Paper III**), two model functions were defined (stroking condition and static brush condition), using the same function with 1 during the 8 s stimulus condition and 0 otherwise. In the analyses of both experiments, model functions were convolved with the canonical hemodynamic response function. The motion parameters and outlier volumes were included in the design matrix as regressors of no interest, and betas for each condition were estimated.

At the group level, the inference about the involvement of brain areas in the investigated processes was performed using statistical parametric t-maps. In each voxel, t statistics were calculated for each condition or contrast between conditions. Parametric approaches have several advantages over nonparametric approaches, e.g. they are more efficient and reproducible (59). However, since it implies one t-test per investigate voxel, the parametric approach is subjected to a risk of false positives and includes some method for multiple comparison correction.

Region of interest analysis for stroking and vibration stimuli in pSTS

Analysis presented in **Paper I** considers processing of tactile stimuli in pSTS. Previous experiments show that this area responds preferentially to the social cues in the visual stimuli such as biological motion. Here, we defined region of interest as a sphere with radius 8 mm at the peak coordinate in the pSTS, reported in the previous paper on processing of skin stroking in posterior temporal lobe (37). Average betas for each condition (stroking and vibration) and each subject were extracted using MarsBaR toolbox, and the analysis of both the level of BOLD responses and the correlation with the pleasantness ratings were estimated.

Processing of stroking and vibration stimuli in insula

The analysis presented in **Paper II** investigated insular processing of stroking and vibration stimuli. Here, we adopted a network approach and focused on the BOLD responses not only in the insula but also in DMN and SN resting state networks. Specifically, we tested the hypothesis that vibration

In addition we investigated functional connectivity within insula using a PPI approach (6), as implemented in SPM8. We used seed regions in posterior and dorsal anterior insula. Dorsal anterior insula is defined as a central hub in SN. In the first step, preprocessed BOLD signal was extracted from the seed regions. In

the second step, a PPI regressor was formed from the interaction between the extracted BOLD signal and the difference between the task regressors (stroking and vibration) convolved with the hemodynamic response function. In the third step, GLM analysis was performed with the PPI regressor, task regressor, and the extracted BOLD signal as the components of the design matrix. The motion parameters and outliers were also included as regressors of no interest. The main effects for the PPI regressor for each subject were estimated. This resulted in one contrast map per subject. At the second level, random effect analysis was used to determine group effect. Statistical parametric maps were investigated within the bilateral insular region of interest.

Processing of skin stroking in patients with AN

In order to investigate differences in brain processing of tactile stimuli in patients with AN, we performed two whole brain analyses. The first analysis focused on the processing of both myelinated and non-myelinated peripheral input from low threshold sensory neurons. Here, we investigated group differences for the main effect of stroking condition. The second analysis aimed to investigate the affective component of the tactile input and examined group differences for the contrast stroking versus static touch.

3.3 Analysis of resting state data

We used the Functional Connectivity (CONN) toolbox (60), implemented in SPM8 to analyse resting state data. CONN uses the CompCor strategy (61) for noise reduction. In the CompCor strategy, the signals from anatomical components (WM and CSF) are used as confounders during the connectivity analysis, in addition to motion parameters and outliers obtained during the preprocessing. Preprocessed data were band pass filtered at 0.008-0.09 Hz. The seed driven analyses were performed by calculating the Pearson's correlation coefficients between the seed's time course and the time course of all other voxels. The correlation coefficients were then converted to normally distributed scores using Fisher's transform, and passed to a group-level GLM analysis.

The first set of resting state data comes from the first experiment. The purpose of seed-to-voxel analysis here was to identify DMN and SN, presented in **Paper II** For construction of the DMN, the centre of the sphere was placed in the posterior cingulate cortex and for construction of the SN the sphere was placed in dorsal anterior cingulate. Both seeds are predefined in CONN.

The aim of the analysis performed on the resting state data collected in the second experiment was to investigate differences in resting state functional connectivity of lateral occipital cortex (LOC) between AN patients and healthy controls. In **Paper III** we unexpectedly found a group difference in this area for the skin stroking condition. In **Paper IV** we present the results from seed-to-

voxel analyses, with seeds in the form of 10 mm spheres placed in left and right LOC at the peak coordinates presented in **Paper III**.

4. Results

4.1 Paper I

The analysis of VAS pleasantness ratings reveals that skin stroking was perceived as pleasant while vibration stimulus was perceived as neutral. Participants perceived stroking as more pleasant than vibration.

Analyses in pSTS region of interest showed no significant activations in this area for stroking and vibration. However, when we performed a correlation analysis between extracted average beta values in this area and pleasantness ratings we found significant positive correlation for stroking. No such correlation was observed for vibration.

We also performed a whole brain exploratory analysis of stroking condition with pleasantness ratings as a covariate of interest and found that the cluster with a peak in the superior temporal gyrus, extending into the pSTS was the largest one.

4.2 Paper II

The analysis of BOLD responses for stroking and vibration within bilateral insula showed that insula is activated both by skin stroking stimulus but also by vibration. In the left insula (contralateral to the stimulated site) both stimuli resulted in the activations in the posterior, middle and anterior parts. In the right insula, stroking activated posterior-middle parts, while vibration activated anterior parts.

The effects of stroking and vibration on DMN were investigated within a mask obtained from the analysis performed on resting state data with the seed placed in the posterior cingulate cortex. This analysis revealed that the levels of activity in four central areas of DMN (ventromedial prefrontal cortex, posterior cingulate cortex and bilateral middle temporal gyrus) were higher for stroking than for vibration. The analysis of extracted beta values in these areas showed that both stroking and vibration in fact resulted in the decreasing activities in DMN compared with the baseline. However this decrease was more pronounced for vibration than for stroking.

We performed two PPI analyses with seeds in the right dorsal anterior insula and in the left posterior insula and estimated results within bilateral insular cortex. For the seed in right dorsal anterior insula, and for the contrast vibration>stroking, one significant cluster was observed in the left middle insula. No clusters were observed for the contrast stroking>vibration. For the seed in the left posterior insula, and for the contrast vibration>stroking, significant clusters were observed in the left middle insula and bilateral dorsal anterior insula. For the contrast stroking>vibration we observed one cluster located in the left ventral anterior insula.

4.3 Paper III

The analysis of pleasantness ratings of skin stroking delivered by LTS showed that healthy controls perceived this stroking as pleasant while in AN patients pleasantness ratings were not significantly different from zero. In addition, we found a significant difference between these two groups' pleasantness ratings.

A whole brain analysis for the main effect of stroking showed activations in a range of areas, including somatosensory areas and the bilateral insula for both groups. For the group difference we found no significant difference in primary and secondary somatosensory cortex, or bilateral insula. However, we found significant group differences in bilateral LOC. An examination of extracted beta values in this area revealed that the observed group difference was a consequence of a weak increase in the activity for healthy controls and a weak decrease in the activity for AN patients.

A whole brain analysis of the contrast stroking versus static touch revealed no group difference in the bilateral insular cortex. The most robust group difference for this contrast was observed in the left caudate nucleus. Here, the extracted average beta values were significantly positive for healthy controls but were not different from zero in AN group. We found no significant correlations between beta values and pleasantness ratings.

4.4 Paper IV

In this paper we investigated resting state functional connectivity of left and right LOC, in healthy controls and in patients with AN. Investigation of the results for each group separately revealed that bilateral LOC displayed significant connectivity with widespread areas in the bilateral occipital and posterior parietal cortex in both groups.

For a group contrast we found significant differences in the medial prefrontal cortex (MPFC) for both left and right LOC. These two regions partially over-

lapped. No other regions were found. When we extracted average beta values in these regions (which here correspond to the correlation of resting state fluctuations between LOC and MPFC) we found that LOC-MPFC connectivities showed different patterns in AN patients and healthy controls, bilaterally. Neither left nor right LOC-MPFC connectivities were significantly larger than zero in healthy controls whereas both were significantly less than zero in patients. We also investigated the correlation between these values and body mass index and found that body mass index correlated positively with left but not right LOC-MPFC connectivity strength in women with AN.

A post hoc reverse analysis with seeds in MPFC showed that resting state connectivity pattern for left MPFC included key regions of the DMN for both AN patients and healthy controls. Group difference for both seeds revealed large clusters centred on the LOC and extending well into the fusiform gyrus.

5. Discussion

5.1 Paper I

We examined the extent to which responses to tactile stimuli in posterior temporal lobe are selective to socio-affective touch by comparing activity elicited by skin stroking and skin vibration. We found that right pSTS responses correlated significantly with participants' perceived pleasantness of skin stroking, but not vibration. These results suggest that right pSTS responses may be selective to skin stroking, but also that pSTS activity is modulated by individual variability in perceived affective quality of touch.

The finding of a correlation between brain responses to skin stroking and pleasantness ratings supports the previously demonstrated link between variability in pSTS processing and social behaviour. We speculate that the demonstrated correlation may reflect a range of individual factors related to socio-affective sensory dimensions, rather than varying levels of peripheral input or low-level processing.

5.2 Paper II

We examined insular processing of discriminative and affective touch by performing extensive analyses of BOLD and functional connectivity responses to skin stroking and skin vibration in insula. We further hypothesised that difference in the cognitive task evaluation between these two tactile stimuli will have impact on the activity level in DMN, and that this effect is to some extent accomplished through the processing of tactile information via cognitive/discriminative path in the insula. Therefore, we extended the analyses also to DMN.

Stroking and vibration were both effective in activating insula. Although the level of activation in somatosensory cortices was higher for stroking than for vibration, vibration decreased the levels of activation in DMN more than stroking. Hence, the stronger decrease of DMN for vibration is probably not a consequence of the intensity of the primary input, but the result of differential higher level processing.

In addition to the posterior insular cortex, both stimuli also activated dorsal anterior insular cortex. Both posterior and dorsal anterior insular cortex displayed

higher functional connectivity with the middle insula for the contrast vibration>stroking. In addition, conjunction analysis on the resulting maps from PPI analyses indicates that posterior and dorsal anterior insular cortex may communicate via middle insula, and that this communication is more pronounced for discriminative touch than for affective touch.

Posterior insular cortex displayed an increase in functional connectivity with the ventral anterior insular cortex for stroking compared to vibration. Thus, the stroking engaged processing in the emotional part of the insula. This is in line with previous studies that identified ventral anterior insular cortex as an area for emotional processing of the sensory inputs (62,63).

Based on these results, we propose a mechanism for processing of discriminative and affective touch in insula. Pleasant (affective) touch is processed through interaction between posterior and ventral anterior insular cortex. On the other hand, descriptive, less emotional, touch is processed through interaction between posterior, middle and dorsal anterior insular cortex. In the dorsal anterior insular cortex, the information reaches SN, which in turn down-regulates the level of activity in DMN depending on the cognitive demand of the sensory input.

5.3 Paper III

We examined brain responses to robot-controlled light skin stroking in AN patients and healthy controls. The lack of group difference in somatosensory brain areas suggests that the basic neural processing of tactile sensations mediated through thick, myelinated A-beta afferents is largely intact in AN. We found an unexpected group difference between healthy controls and AN patients in the LOC, an area not typically involved in tactile processing. Instead, LOC is critically involved in the processing of images of human bodies (64–66) and in self-representation (64). Studies of visual perception of human bodies show alterations in LOC processing in AN (67,68). In addition, LOC exhibits altered functional connectivity in AN (51,68,69). We suggest the observed LOC difference may be a consequence of the dysfunction in the body perception network in AN. Further research is required to elucidate the underlying cause of these findings.

AN patients rated skin stroking as less pleasant than healthy controls, which confirms previous results (57). The contrast stroking versus static touch did not reveal any alterations in the processing of affective touch in insula. However, we found the largest group difference in the left caudate nucleus. Previous studies showed that alterations in the dorsal striatum are associated with the shaping of maladaptive eating behaviour in AN and are also reflected in the alterations to the reward and punishment in monetary task paradigms (70,71). The dysfunctional processing of skin stroking stimuli in the brain reward system may be an explanation to the decreased pleasantness percept of gentle skin stroking in AN.

5.4 Paper IV

We examined LOC connectivities in AN patients, and found that LOC-MPFC functional connectivity was altered in patients relative to healthy controls. Specifically, we observed bilateral negative LOC-MPFC connectivity in women with AN, that was not present in healthy controls. Moreover, left LOC-MPFC functional connectivity was positively associated with body mass index in AN patients.

We found AN-specific functional couplings between the LOC and the MPFC. The MPFC is associated with self-related processing (72), emotionally significant information (73), stress (74) and emotion regulation (75). Our results suggest that the LOC may exert abnormal influences on emotionally valenced self-related processing in AN. Specifically, we speculate that the LOC-MPFC coupling may underpin the strong association between self worth and body weight characteristic of AN.

The LOC-MPFC connectivity was negative in AN patients; hence any dysregulatory effects involve increased LOC and decreased MPFC activity, and vice versa. This effect may be related to the DMN: DMN activation is specifically associated with a range of introspective processes, such as self-referential processing (76,77), self-reflection (78) and self-knowledge (79), whereas DMN suppression is linked to external stimuli, tasks or anxiety (80). Hence, high LOC activity during rest may modulate the MPFC, which in turn may suppress the DMN. A connection between the LOC and a key node of the DMN may explain the relentless thoughts on their own body typical in AN.

The strong LOC-MPFC connectivity may reflect an abnormal influence of body processing on the patients' mental states. This finding is in line with the previous studies of Friederich et al. (81,82). Specifically, Friederich and co-workers showed that, in healthy women, body sensitive areas including the LOC were activated during comparison of ones own body with that of model women in magazines, and that MPFC activity correlated positively with experienced anxiety (81). In light of this observation, we hypothesize that a body-self network transiently evoked in healthy women during explicit comparisons between others' bodies and ones own, is incorporated into the resting state networks in women with AN. Alternatively, AN patients may be actively contemplating body-related information during rest. Our results support the hypothesis that MPFC processing may mediate the negative impact of self-comparisons with idealized media body images (81).

Our findings have a number of important implications. First, the results provide a possible neural mechanism for body fixation in AN. Second, the LOC-MPFC connectivity constitutes a promising potential biomarker of AN, as it shows specificity on the individual level and correlates with BMI. Third, the LOC-MPFC circuit may be a treatment target. Disrupting the LOC-MPFC connectivi-

ty may reduce the aberrant influence of body shape and weight on self-processing and accelerate recovery. Such disruption may be achieved using neuromodulation techniques (83), such as transcranial magnetic stimulation (TMS). The LOC-MPFC connectivity may serve as a biological marker, indicating both who may benefit from neurostimulation treatment as well as to directly follow how effective the treatment is.

6. Conclusions

Paper I

The posterior temporal lobe has an important role in processing socio-affective dimensions of touch. Specifically, our study supports the notion that socio-affective touch may be selectively processed in the temporal lobe; however, our results also suggest that any selectivity is contingent on top-down effects related to subjectively perceived qualities of the tactile stimulation.

Paper II

The insula processes affective touch information and also information about touch with less emotional valence. We suggest that pleasant (affective) touch is processed through interaction between the posterior and ventral anterior insular cortex. On the other hand, descriptive, less emotional touch is processed through interaction between posterior, middle and right dorsal anterior insular cortex. Here, the information reaches SN, which in turn down-regulates the level of activity in DMN depending on the cognitive demand of the sensory input.

Paper III

AN patients perceived skin stroking as less pleasant than healthy controls, which might be due to the dysfunction in their brain reward system (caudate nucleus). We also found an alteration in the BOLD response in LOC during tactile stimulation, and suggest that this part of the brain may play a central role in the disturbed body image perception in AN

Paper IV

We found that body sensitive LOC is functionally connected to an introspective brain region in AN patients but not in healthy controls. Hence, the strong LOC-MPFC connectivity may reflect an abnormal influence of body processing on the patients' mental states.

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